Testing how methods impact the results of interspecific competition research

Esther Woo, Lin Jiang

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Abstract

There are currently two methods that can be used to measure interspecific competition, pairwise and mutual invasion. Both can be used to generate niche difference (ND) and relative fitness difference (RFD) values, as well as determine if two species are able to coexist. Although the newer method, mutual invasion, has been in use for eight years, there has not yet been a study that compares the two. In order to determine if the method impacts the results found in a study, two simple experiments were conducted concurrently. The five-week long experiment involved determining whether *Colpidium striatum* and *Tetrahymena pyriformis* are able to coexist. Upon completion, both methods concluded that they could coexist. Despite reaching the same conclusion, it is still unknown if other species pairings or more complex experiments would alter these results.

Introduction

In ecological research, it is important to accurately measure interspecific and intraspecific competition to determine if different species can coexist with each other. In order for two species to occupy the same space, the competition that occurs within the same species must exceed the

competition between the two different species. Currently, when measuring niche and relative fitness difference for interspecific competition, there are two main methods. One is the traditional pairwise method that uses the Lotka-Volterra model which is part of a series of equations that can be used to depict the dynamics of biological systems (Morris & Pratt, 2003). In 2011 a newer method (Carroll et al. 2011) was introduced that can be used to calculate the same values as the pairwise method. Since no study has directly compared the results of the two methods to see if they are precise, it is unknown if one or either method is accurate.

Some studies begin with immediate pairwise competition which is adding multiple species to an environment (a microcosm, plot, or agar) simultaneously. This method looks at the how species coexist with direct competition (Gotelli 1999, Violle et al. 2011, Narwani et al. 2013, Li et al. 2016). Others involve mutual invasion which means there is a lag between adding the first species and any subsequent species to the given environment. This is used to see how the species' interactions are affected by colonization history (Pu and Jiang 2015, Johnston et al. 2016, Ojima and Jiang 2017). Studies using this method have found that community assembly does have a significant impact on species productivity (Brook et al. 2003) and response to adaptive radiation (Tan et al. 2017). With this effect, it is possible that previous studies not accounting for community assembly may have had a different result if they had.

No studies exist that directly compare the results for these two methods. Distinguishing if there is a difference between the results of the two methods is important for determining what variables do indeed affect interspecific competition. This is required to ensure that there are no unknown third variables affecting research results. We look to separate the differences in relative fitness in species: niche difference and fitness difference (Carroll et al. 2011). We are doing a five-week long experiments, simultaneously running each method, using ciliate protozoan species

Tetrahymena tyriformis and *Colpidium striatum*. The goal is to determine if the methods affect the evaluation of competitive ability and if they skew results.

Literature Review

Competition is a form of negative symbiosis, that is, organism interaction with a negative impact for one or both individuals involved. Typically, these interactions are based around a lack of resources like food or territory. Competition may be intraspecific, between individuals of the same species, or interspecific, between two or more different species' populations. Interspecific competition makes up a major part of ecological interactions, and is often used as a model for environmental response to stressors like environmental change (Fox and Morin 2001) and overall density dependence (Jiang and Shao 2003).

Interspecific competition is typically measured in one of two ways. In the first method, the same number of individuals of each species is introduced to an environment simultaneously. In the second method, there are two treatments. In treatment one, one species' population grows to carrying capacity, which is the maximum population size allowed according to the amount of resources available for a species, before adding the other species. In treatment two, the exact same is done but in reverse order of species addition.

From here on, these methods will be referred to as pairwise competition and mutual invasion respectively. The pairwise method has been used in the past to look at multiple competitive situations, such as which species dominates after new niche openings (Li et al. 2016), competitive response to temperature change (Fox and Morin 2001), how competition affects extinction rates during high disturbance (Violle et al. 2010), and how evolutionary relatedness affects coexistence (Narwani et al. 2013) and competition (Violle et al. 2011). Studies using this

method tend to hypothesize how competition is affected by outside factors. Whereas, studies using mutual invasion are specifically seeing how community assembly order affects species response to outside factors like niche openings (Brook et al. 2003), to different disturbance levels (Ojima and Jiang 2017), and to adaptive radiation (Tan et al. 2017). Another study evaluates the significant effect colonization history has on the final metacommunity (Pu and Jiang 2015), which is the sum of all interacting habitats.

Though many studies have used one of these methods, no study has used both to ensure precision. Without such research, there is no clear indication of whether using a different method in these studies would result in a different conclusion. In order to evaluate this, we have measured the growth rate and ratio between two different species over a month for pairwise competition and mutual invasion methods. The data was evaluated using Lotka-Volterra (Morris and Pratt 2003) and the invader-native method (Li et al. 2019). These results will indicate if colonization history should be considered when measuring differences in niche and competitive ability or if the results are similar enough to disregard the mutual invasion method, which is more complicated and time intensive.

Methods

We selected the protozoan species *Colpidium striatum* (C) and *Tetrahymena pyriformis* (T) for their easy distinctions in size and generation time. Cultures were made with 100ml of bacterialized .55pp stock in a 250ml jar. The stock was made using protozoan pellets.

Experimental Treatment

Two monocultures, *C. striatum* (C) and *T. pyriformis* (T), and one mixed culture of both species (CT) were made by adding 100 individuals of each species to a culture. The mixed culture had 5 replicates and the monocultures had 10 replicates to account for the ones needed later for the mutual-invasion method. After setting up, each culture was counted three times a week for a density (organisms/1mL). After 2 weeks, half of the monoculture replicates had 100 individuals of the other species added (T<C, C<T). All resulting cultures were counted for another three weeks. The original biculture (CT) served as the pairwise method and the species with another added after 2 weeks (T<C, C<T) were the mutual invasion method with the remaining monocultures as controls.

Protist Counting

The 8-drop method was consistently used for finding the concentration of organisms per milliliter of solution. This involved measuring about .3 mL of the stock solution containing the organism into 8 droplets. The number of individuals (C) was counted then divided by the exact

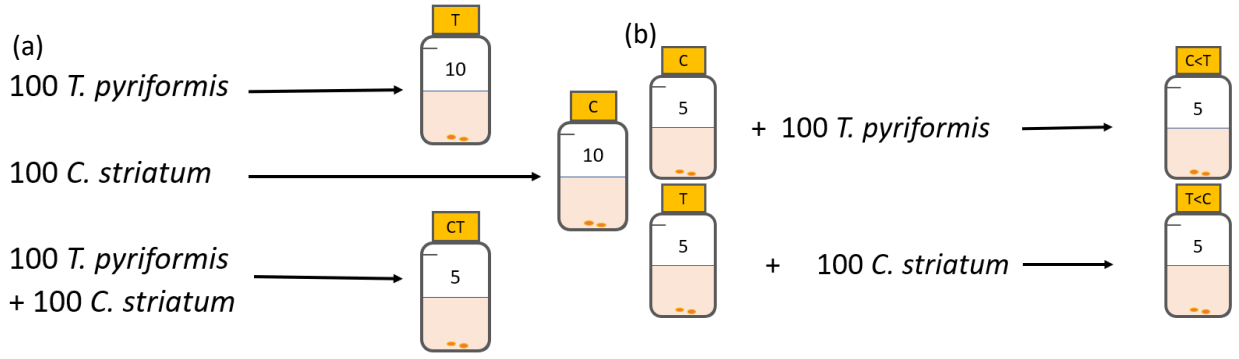

Figure 1. Experimental Design. The figure above visualizes the experimental design. (a) depicts the Day 0 cultures, there are 25 in total. The final row of 5 replicates serve as the pairwise method treatment. (b) depicts the mutual invasion method treatment that was done by adding a second species to half of the monocultures on day 14 of the experiment. The remaining monocultures served as the controls throughout the 5-week experiment.

volume of the stock used (D) to find the estimated density of organisms in the stock solution (per mL):

$$\frac{C}{D} = number\ of\ organisms/mL$$

If the stock needs to be diluted, then the total volume after dilution (D2) should be divided by the initial volume (D1). This value should be multiplied by the count of the organism (C) divided by the volume taken from the dilution (D3):

$$\frac{D_2}{D_1} \times \frac{C}{D_3} = number\ of\ organisms/mL$$

It is important to note that in cultures including both species, the *C. striatum*'s population size was counted before diluting for the higher population species. In a preliminary version of this experiment, when this had not been done, the measured density for *C. striatum* was not accurate after diluting.

Niche and Relative Fitness Difference Calculations

In the pairwise method, the monocultures were used to find each species' average α value, which is the negative effect on the total species population caused by individuals of the same species. The bicultures were then used to find each species' competition coefficient effect, α_{ij} and α_{ji}, which is the negative effect of the second species in the subscript on the first. These values were then used to find a niche difference (ND) and relative fitness difference (RFD) for each replicate using the following equations based on Lotka-Volterra (Godoy et al. 2014).

$$ND = 1 - \sqrt{\frac{\alpha_{ij}\alpha_{ji}}{\alpha_{ii}\alpha_{jj}}}$$

$$RFD = \sqrt{\frac{\alpha_{ji}\alpha_{jj}}{\alpha_{ij}\alpha_{ii}}}$$

For mutual invasion, the per capita growth rates of each species' monoculture (μ_{alone}) were compared to the per capita growth rate when invading another species for each species combination ($\mu_{invading}$).

$$S = \frac{\mu_{alone} - \mu_{invading}}{\mu_{alone}}$$

These species (S) values were then used to calculate the niche difference and relative fitness difference (Li et al. 2019).

$$ND = 1 - \sqrt{S_i S_j}$$

$$RFD = \sqrt{\frac{S_j}{S_i}}$$

Statistical Tests

The resulting niche difference and relative fitness difference for each species were compared between the two methods. This was done with a two-tailed independent unequal variance t-test ($\alpha = 0.05$). The test used was independent because the results of each study were not impacted by the results of the other, and a t-test was used in order to determine if there was a significant difference in results of the two methods.

Evaluating Coexistence

In addition to statistically comparing the ND and RFD values, the resulting data was used to determine if the species *C. striatum* and *T. pyriformis* are able to coexist with each other in order to provide a qualitative comparison of the two methods. For the pairwise method this was done

using Zero Net Growth Isoclines (ZNGI) which involved graphing each species using their carrying capacity (K_i) and carrying capacity over alpha (K_i/α_{ij}) (Morin 2011). The mutual invasion method used the following equation as a boundary of competitive exclusion (Narwani et al. 2013):

$$RFD = (1 - ND)^{-1}$$

Results

The data found in this experiment was measured quantitatively and qualitatively with mixed results. After comparing the population of each species in each treatment for both methods, it was found that there was a statistically significant difference between the ND (p = 0.0252) and RFD (p = 0.0179) values found using both methods.

In the ZGNI test, the $K_T < K_C/\alpha_{CT}$ and $K_C < K_T/\alpha_{TC}$ which indicates that *C. striatum* and *T. pyriformis* are able to coexist with each other according to the pairwise method. The mutual invasion showed that the RFD was greater than $(1 - ND)^{-1}$ which also indicates that they can coexist.

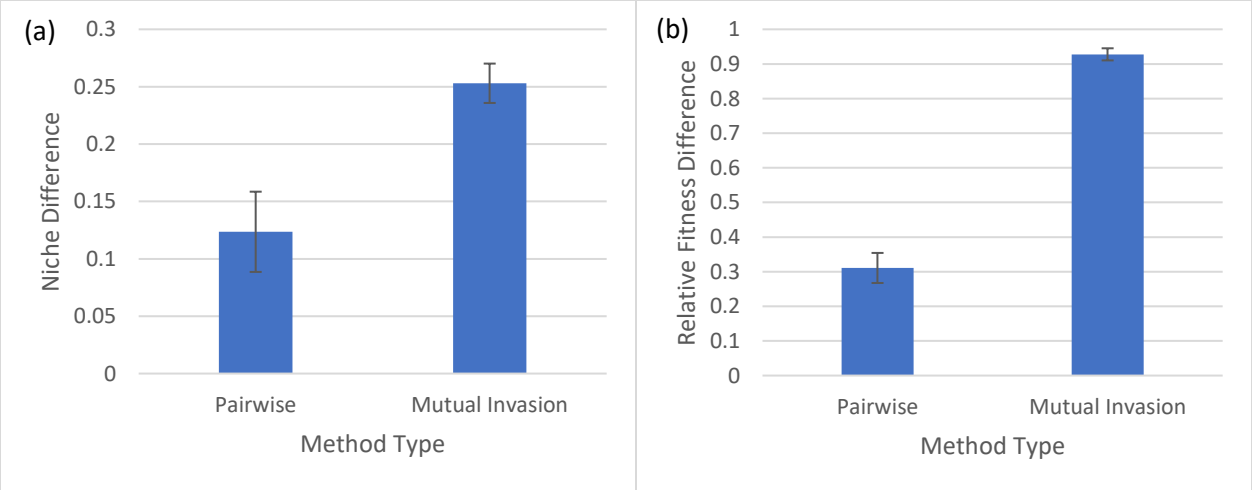

Figure 2. Niche differences (ND) and relative fitness differences (RFD) for both methods. The figure above compares the values found with both methods. (a) shows that the mutual invasion method's ND is significantly greater than the pairwise's (p = 0.0252). (b) also shows that mutual invasion's RFD is significantly greater than pairwise's (p = 0.0179).

Discussion

The original intent of the study was to directly compare the niche difference (ND) and relative fitness difference (RFD) values of the two methods. There is a significant difference in the ND and RFD values found with each method. Despite this, these values while accurate relative to each other within the same model, should not be directly compared between different models. Though both experiments agreed that *C. striatum* and *T. pyriformis* can coexist, it not conclusive that these methods always create the same results. While both are at equilibrium, it is unknown if this is stable or unstable, or if this conclusion will remain the same under different conditions and species combinations.

Future studies that include more variables and different species will help determine if these two methods are comparable to each other. Repeating this experiment with species pairings that are known to have higher or lower ND and RFD values may lead to the conclusion that a certain difference is the threshold between the method impacting the results. Adding stressors like temperature, resource competition, predation, or sonication may also cause the results to be dependent on the method used.

Based on the results of this study, it may appear that the pairwise and the mutual invasion method are alternatives to reach the same results. It is simpler to prove that the pairwise and mutual invasion methods end in different results than that the two methods are interchangeable. Repeated studies that consider the factors that impact the ability for coexistence need to be considered before the latter can be definitively stated.

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